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Epistatic Interactions Between *smell-impaired* Loci in *Drosophila melanogaster*

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ABSTRACT

Odor-guided behavior is a polygenic trait determined by the concerted expression of multiple loci. Previously, *P*-element mutagenesis was used to identify single *P[lArB]* insertions, in a common isogenic background, with homozygous effects on olfactory behavior. Here, we have crossed 12 lines with these *smell impaired* (*smi*) mutations in a half-diallel design (excluding homozygous parental genotypes and reciprocal crosses) to produce all possible 66 doubly heterozygous hybrids with *P[lArB]* insertions at two distinct locations. The olfactory behavior of the transheterozygous progeny was measured using an assay that quantified the avoidance response to the repellent odorant benzaldehyde. There was significant variation in general combining abilities of avoidance scores among the *smi* mutants, indicating variation in heterozygous effects. Further, there was significant variation among specific combining abilities of each cross, indicating dependencies of heterozygous effects on the *smi* locus genotypes, *i.e.*, epistasis. Significant epistatic interactions were identified for nine transheterozygote genotypes, involving 10 of the 12 *smi* loci. Eight of these loci form an interacting ensemble of genes that modulate expression of the behavioral phenotype. These observations illustrate the power of quantitative genetic analyses to detect subtle phenotypic effects and point to an extensive network of epistatic interactions among genes in the olfactory subgenome.

THE fundamental goal of quantitative genetics is to understand how complex traits are shaped through the interactions of multiple genes in different genetic backgrounds and under varying environmental conditions. Perhaps the most complex category of polygenic traits is represented by various forms of animal behavior. *Drosophila melanogaster* presents an ideal model system to study the genetic basis of behavioral quantitative traits, because mutations in highly inbred strains can be easily generated, allowing control over the segregation of many individual loci that contribute to the trait and enabling the effect of each locus to be studied independently. We have used odor-guided behavior in *D. melanogaster* as a model system to study the quantitative genetics of behavior.

Odor-guided behavior is of special interest, because the ability of an organism to respond to chemical signals from its environment is essential for its survival and, often, its procreation. Thus, olfactory behavior contributes to individual fitness (MACKAY *et al.* 1996). In recent years, considerable progress has been made in elucidating the molecular mechanisms that underlie odor recognition, olfactory transduction, and neural coding of olfactory information both in vertebrates (reviewed by ANHOLT 1993; AXEL 1995; BUCK 1996) and in invertebrate model systems, such as *Caenorhabditis elegans* (TROEMEL *et al.* 1996; SENGUPTA *et al.* 1996) and lobster (FADOOL and ACHE 1992). However, the genetic basis of variation in olfactory responsiveness and the genetic mechanisms that shape behavioral responses to odorants are still poorly understood.

Chemical mutagenesis has been used to induce mutations affecting olfactory behavior in *D. melanogaster*, mostly on the *X* chromosome (RODRIGUES and SIDDIQI 1978; ACEVES-PIÑA and QUINN 1979; HELFAND and CARLSON 1989; LILLY and CARLSON 1989; MCKENNA *et al.* 1989; AYER and CARLSON 1992; WOODARD *et al.* 1992; LILLY *et al.* 1994a,b). This resulted in the characterization of a number of genes that encode proteins likely to participate in olfactory signal transduction in Drosophila, such as *smellblind* (an allele of *paralytic*), which encodes a voltage-gated sodium channel (RODRIGUES and SIDDIQI 1978; ACEVES-PIÑA and QUINN 1979; LILLY and CARLSON 1989; LILLY *et al.* 1994a,b), *norpA*, which encodes a phospholipase C (WOODARD *et al.* 1992; RIESGO-ESCOVAR *et al.* 1995), and *rdgB*, which encodes a phosphatidyl inositol transfer protein (VIHTELIC *et al.* 1993). Although mutations in any of these genes cause extensive impairment of olfactory behavior, it is not clear how these genes contribute quantitatively to variation in olfactory responsiveness and how they function in the context of the genetic background, *i.e.*, the entire olfactory subgenome. Recently, we have identified 14 loci that contribute to olfactory behavior by *P*-element insertional mutagenesis

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in an isogenic strain (ANHOLT *et al.* 1996). Identification of these loci, designated *smell impaired* (*smi*), was achieved using statistical and quantitative genetic analysis of measurements of olfactory behavior. These analyses are capable of detecting small phenotypic effects with a resolution limited only by sample size.

As the *smi* loci have similar phenotypes, they are likely to be functionally related and participate in common physiological and/or developmental pathways that shape olfactory responsiveness. One genetic method for identifying and ordering genes in functionally interacting groups is to screen for mutations at unlinked loci that enhance or suppress the mutant effects of a known member of the pathway (GARCÍA-BELLIDO 1981). Epistatic interactions between such genes can be deduced by examining the phenotypes of the one- and two-locus genotypes. For independent loci, the phenotypes of the two-locus genotypes are the sum of the single-locus phenotypes; *i.e.*, the loci act additively. Departures from strict additivity indicate epistatic, or interacting, loci. A simple test for interaction that can be used for recessive mutations with large, qualitative effects that have similar loss-of-function phenotypes and that therefore affect a common process, is to examine the phenotypes of double mutant heterozygotes. Epistasis is evident when the double heterozygote has the same loss-of-function phenotype as the single homozygous mutations as a result of combined haploinsufficiency of function. This approach and variants of it have been used to identify epistatic interactions and to identify new loci that modify mutant phenotypes of other loci (BOTAS *et al.* 1982; BELOTE *et al.* 1985; KENNISON and RUSSELL 1987; HOMYK and EMERSON 1988; TRICOIRE 1988; DAMBLY-CHAUDIÈRE *et al.* 1988).

Detecting interactions between mutations with quantitative effects is more difficult, because the mutations are not usually completely recessive (MACKAY *et al.* 1992; LYMAN *et al.* 1996). Further, the background genotype needs to be controlled to enable small phenotypic effects to be perceived and to ensure any interactions are due to epistasis between the mutations of interest, and are not confounding nonadditive interactions among alleles segregating between the background genotypes in which the mutations were induced. The *smi* mutations are in a common isogenic background and therefore can be used to detect epistasis. We have generated all possible double heterozygous hybrids among 12 independent *smi* mutations that appear amenable to molecular characterization in a diallel cross design (GRIFFING 1956), which is the quantitative genetic analogue of the transheterozygote test for epistasis. This approach is based on the assumption that reduced expression of two independent *P[lArB]*-tagged *smi* genes in double heterozygotic offspring may result in quantitative failure to complement (MACKAY and FRY 1996; LONG *et al.* 1996) if these genes interact.

Significant epistatic interactions were identified for nine transheterozygote genotypes, involving 10 of the 12 *smi* loci. Interactions between eight of these loci show evidence of a web of mutually interactive genes, the coordinated expression of which modulates the behavioral phenotype. These findings illustrate the power of quantitative genetic analyses to detect subtle phenotypic effects and indicate that phenotypic determination of odor-guided behavior in *D. melanogaster* depends quantitatively on an extensive network of genetic interactions.

MATERIALS AND METHODS

Generation of transheterozygous *P[lArB]* insert lines: The parental lines used to generate double mutant heterozygotes were 12 homozygous *smi* lines obtained by *P*-element mutagenesis of the isogenic *Samarkand; ry*^{506} strain: *smi21F, smi26D, smi27E, smi28E, smi35A, smi45E, smi51A, smi60E, smi61A, smi65A, smi97B,* and *smi98B* (ANHOLT *et al.* 1996). The mutations were named according to the cytological insertion sites of the *P* elements. The 12 *smi* lines were crossed in a half-diallel design (excluding homozygous parental lines and reciprocal crosses) to produce all 66 possible combinations of F_1 transheterozygous offspring with two *P* elements at different loci. Crosses were initiated at a density of five females of *smi* line *i* and five males of *smi* line *j* ($i \neq j$) in plastic culture vials. All animals were reared at 25° on agar-yeast-molasses medium.

Behavioral assay: To quantify odor-guided behavior we used the simple, rapid, and highly reproducible "dipstick" assay, described previously (ANHOLT *et al.* 1996). This assay was chosen because it has several advantages over other commonly used assays. The Y-maze assay, developed by RODRIGUES and SIDDIQI (1978), is better suited for measurements of attraction and odor discrimination than repulsion and is laborious for large behavioral screens. The olfactory jump assay, described by MCKENNA *et al.* (1989), was in our hands unreliable, because in contrast to Canton-S flies for which this assay was developed, flies of both our inbred Samarkand strain and of substitution lines containing chromosomes from natural populations (MACKAY *et al.* 1996) seldom jumped in response to repellent odorants. The "dipstick assay" used in this study and in previous studies (ANHOLT *et al.* 1996; MACKAY *et al.* 1996) is a simple, rapid, and highly reproducible statistical sampling assay that quantifies odor-guided behavior with a resolution limited only by sample size and, hence, can detect subtle olfactory impairments (ANHOLT *et al.* 1996). Previously, this assay has led to the identification of 14 novel *smi* loci. In addition, known mutants, such as *smellblind* (RODRIGUES and SIDDIQI 1978; ACEVES-PIÑA and QUINN 1979; LILLY and CARLSON 1989; LILLY *et al.* 1994a,b), are immediately apparent and readily quantifiable in this assay (ANHOLT *et al.* 1996).

After 2–4 hr of starvation, 2–10-day post-eclosion transheterozygous progeny were tested for responsiveness to benzaldehyde, a repellent odorant, exactly as described by ANHOLT *et al.* (1996). Briefly, one replicate assay consisted of a single-sex group of five individuals in a test vial. The animals were exposed to 1% benzaldehyde (v/v) introduced on a cotton wool swab, and the number of flies migrating to a compartment remote from the odor source was measured at 5-sec intervals, from 15 to 60 sec after introduction of the odor source. The "avoidance score" of the replicate is the average of these 10 counts, giving a possible range of avoidance scores between 0 (all flies in the compartment near the odor source for the entire assay period) and 5 (all flies in the compartment away from the odor source for the entire assay period). For each of the 66 crosses, 10 replicate avoidance score estimates were obtained for each sex, for a total of 20 replicates (100

individual flies) per double heterozygote genotype, and a total sample size of 6600 animals.

Statistical analyses: The avoidance scores of transheterozygous genotypes were analyzed by two-way analysis of variance (ANOVA), with Genotype and Sex the fixed cross-classified main effects. Sums of squares were partitioned into sources (degrees of freedom) attributable to Genotype (65), Sex (1), Genotype × Sex interaction (65), and Error (1188). As this is a fixed effects model, the error mean square was used as the denominator for all *F*-ratio tests of significance. To analyze epistatic effects between *smi* loci, we could not simply compare the responses of double heterozygotes with the single heterozygotes of *smi* lines with *Sam*, because the effect of *P[lArB]* insert copy number (2 *vs.* 1) could be confounding. Rather, the correct control for this analysis is measurement of the deviation from the average of all other transheterozygotes with the two single inserts being compared. Thus, the general combining ability (*GCA*) of a mutation is its average avoidance score as a transheterozygote with all other mutations, expressed as the deviation from the overall mean (SPRAGUE and TATUM 1942), and is an estimate of the average heterozygous effect of the mutation relative to the heterozygous effects of the other mutations. The specific combining ability (*SCA*) of a transheterozygous genotype is the difference between the observed avoidance score of the genotype, x_{ij} (where i and j denote two different *smi* mutations), and the score expected from the sum of the corresponding *GCA*s of mutants i and j. The sums of squares due to Genotype and Genotype × Sex were further partitioned into sources of variation (degrees of freedom) attributable to *GCA* (11), *SCA* (54), *GCA* × Sex (11), and *SCA* × Sex (54). *SCA* effects are due to variation in heterozygous effects that depend on the genetic background with respect to other *smi* mutations and can only be caused by epistatic interactions.

This fixed effects half-diallel corresponds to Method 4, Model I of GRIFFING (1956). Consequently, the *GCA* for each *smi* mutant was estimated as

$$GCA_I = T_i/(n-2) - \sum T/n(n-2) \qquad (1)$$

where T_i is the sum of mean avoidance score values (averaged over all replicates) of heterozygotes with the ith mutation, ΣT is twice the sum of mean avoidance score values of all heterozygotes, and n is the number of mutant lines (see also FALCONER and MACKAY 1996). The *SCA* effects were computed using the method of GRIFFING (1956) for each heterozygous genotype as

$$SCA_{ij} = x_{ij} - (T_i + T_j)/(n-2) + \sum T/(n-1)(n-2). \quad (2)$$

The significance of the overall *GCA*, *SCA*, *GCA* × Sex, and *SCA* × Sex effects was tested using an *F* variance ratio test statistic with the error mean square as the denominator. Standard errors of individual *GCA* and *SCA* effects were computed according to the formulae given by GRIFFING (1956). Analyses of variance and tests of significance were calculated using SAS procedures (SAS INSTITUTE, INC. 1988), and *GCA* and *SCA* sums of squares were computed using the diallel cross analysis program of SCHAFFER and USANIS (1969).

RESULTS AND DISCUSSION

The effects of 12 *P*-element insertional mutations with homozygous effects on olfactory behavior were evaluated in all possible double heterozygote combinations, in a half-diallel design. The mean avoidance responses to benzaldehyde, averaged over sexes, are shown for each of the 66 transheterozygote genotypes in Table 1.

TABLE 1

Diallel cross of the *smi* lines

	smi 97B	*26D*	*51A*	*27E*	*60E*	*35A*	*45E*	*61A*	*65A*	*28E*	*21F*	T_i	*GCA*	*HOM*
98B	3.640	3.365	3.800	3.870	3.600	4.020	4.085	4.435	3.985	4.160	4.145	43.105	−0.083^{ns}	2.870
97B		3.510	3.960	3.805	3.810	4.060	4.250	4.330	3.925	3.800	3.520	42.610	−0.133**	1.975
26D			3.355	3.935	3.655	3.710	3.790	4.010	3.655	3.690	4.055	40.730	−0.321***	2.600
51A				3.825	3.720	4.020	4.070	4.320	3.510	3.890	3.700	42.170	−0.177***	3.220
27E					3.890	4.105	4.025	4.340	3.920	3.695	4.300	43.710	−0.023^{ns}	2.230
60E						4.385	4.355	3.810	3.945	4.190	3.980	43.340	−0.060^{ns}	2.270
35A							4.515	4.680	4.390	4.375	4.290	46.550	0.261***	3.190
45E								4.480	4.240	4.130	3.720	45.660	0.172***	3.155
61A									4.145	4.260	4.170	46.980	0.304***	3.210
65A										3.970	4.135	43.820	−0.012^{ns}	2.930
28E											4.200	44.360	0.042^{ns}	2.540
21F												44.215	0.028^{ns}	3.135

Flies of each of 12 *smi* lines, with mean homozygous avoidance scores as given in the last column (ANHOLT *et al.* 1996), were crossed to flies of the remaining 11 lines. The parental *smi* lines are listed in the top row and first column. The arithmetic means of avoidance scores from 20 measurements are given for each hybrid cross. T_i is the sum of avoidance scores used to compute the *GCA* for each line. *GCA* is defined in the text. Avoidance scores of the parental homozygous *smi* lines (*HOM*) are given for comparison (ANHOLT *et al.* 1996). Avoidance scores for males and females of sexually dimorphic *smi* lines (*smi21F*, *smi45E*, *smi51A*, and *smi97B*; ANHOLT *et al.* 1996) are averaged, because no statistically significant sex-specific epistatic effects were observed for the transheterozygotes derived from these lines. $^{ns}P > 0.05$, not significant; $^{**}0.001 < P < 0.01$; $^{***}P < 0.0001$.

TABLE 2

Analysis of variance of avoidance responses to benzaldehyde of transheterozygous *smi* lines

Source	d.f.	SS	*F*	*P*
Genotype	65	110.917	3.82	0.0001
Sex	1	4.983	11.15	0.0009
Genotype × Sex	65	32.493	1.12	0.2457
Error	1188	530.695		

TABLE 3

Analysis of variance of general and specific combining abilities of transheterozygous *smi* lines

Source	d.f.	SS	*F*	*P*
Sex	1	4.983	11.15	0.0009
GCA	11	71.189	14.49	<0.0001
SCA	54	39.728	1.65	0.0025
GCA × Sex	11	2.711	0.55	0.87
SCA × Sex	54	29.782	1.23	0.13
Error	1188	530.695		

The analysis of variance of these data is given in Table 2. The differences in mean avoidance responses among the heterozygous genotypes were highly significant ($P = 0.0001$). There was also significant sexual dimorphism in avoidance response to benzaldehyde, averaged over all genotypes ($P = 0.0009$), with a mean male avoidance score of 4.1 and a mean female score of 3.9. Sexual dimorphism for olfactory avoidance response has been observed previously for homozygous *P*-element insertional mutations (ANHOLT *et al.* 1996) and among a sample of isogenic *X* and third chromosomes extracted from a natural population and substituted into the same inbred strain used for *P*-element mutagenesis (MACKAY *et al.* 1996). Interestingly, both the homozygous *P*-element insertions and the naturally occurring alleles affecting olfactory behavior had very large genotype × sex interaction effects, indicating that there was variation in the magnitude of the sex dimorphism of effects among the homozygous genotypes. However, the genotype × sex interaction was not significant for the double heterozygote genotypes; therefore, the sex-specific effects observed previously are on average recessive.

Variation among the transheterozygous genotypes can arise from two sources: variation in mean heterozygous effects of the different mutations, and variation from epistatic interactions. Because all *P*-element insertions are in the same inbred strain, all genetic variation among the genotypes is attributable to one of these two sources, with no confounding effects contributed by the background genotype. Classical diallel cross analysis enables us to separate heterozygous from epistatic effects by partitioning the variation among double heterozygous genotypes into their general (*GCA*) and specific (*SCA*) combining abilities. As mentioned above, the *GCA* of a mutation is an estimate of its mean heterozygous effect in the background of each of the other mutations. Estimates of the *GCA* of each *smi* mutation, expressed as deviations from the overall mean of the population of heterozygous genotypes, are given in Table 1. For comparison, also given in Table 1 are the mean avoidance scores of each *smi* mutation, at the same concentration of odorant used to assess transheterozygote olfactory behavior (*HOM*; ANHOLT *et al.* 1996). All homozygous *smi* mutations have reduced avoidance scores relative to the transheterozygotes. Therefore, negative *GCA* effects reflect lower mean heterozygous avoidance scores and a more mutant heterozygous phenotype; conversely, positive *GCA* effects reflect higher than average mean heterozygous scores and a more wild-type phenotype. This variation in *GCA* among the *smi* mutations is highly significant ($P < 0.0001$, Table 3), and from this we can infer that all the *smi* mutations are not completely recessive.

The overall mean avoidance score of the transheterozygous genotypes, 3.99 ± 0.08 (Table 1), is significantly higher than that of the *Sam; ry*^{506} strain, 3.65 ± 0.08 (ANHOLT *et al.* 1996). Technically, this could be interpreted as overdominance for olfactory behavior. However, the selectable visible marker used in this system of *P*-element mutagenesis is *ry*$^{+}$, and there is a concern that this marker has a direct effect on fitness and other quantitative traits relative to the *ry*$^{-}$ mutant background of the control strain (LYMAN *et al.* 1996). For this reason, we cannot use these data to estimate *d*, the value of the heterozygote expressed as a deviation from the mean mutant and control strain value (FALCONER and MACKAY 1996) for each *smi* mutation. However, we can estimate the average degree of dominance of the *smi* mutations from the slope of the regression *b* of *GCA* on homozygous avoidance score of *smi* mutations, where each homozygous score is expressed as a deviation from the overall homozygous mutant mean. The estimate of the average degree of dominance *k* is $2(b - 0.5)$ (MACKAY *et al.* 1992; LYMAN *et al.* 1996), where *k* ranges from −1 (completely recessive mutations) through 0 (strict additivity) to 1 (completely dominant mutations). For these data, $b = 0.197$ ($0.01 < P < 0.05$) and $k = -0.605$. On average, the *smi* mutations are partially recessive.

The *SCA* of a pair of mutations reflects the extent to which the mean avoidance score of the double heterozygote, expressed as a deviation from the mean of the total population of heterozygous genotypes, departs from that expected given the sum of the *GCA*s of the two mutant parents. Typically, diallel crosses are made among inbred lines that each vary at a number of loci affecting the measured trait, and significant *SCA* effects can only be attributed to nonadditive interactions in general, including dominance and epistasis (FALCONER

TABLE 4

Estimates of specific combining abilities of transheterozygous *smi* lines

Parent 1	Parent 2	*SCA*	*P*
smi21F	*smi26D*	0.354	0.009
smi21F	*smi27E*	0.301	0.026
smi21F	*smi45E*	−0.474	0.001
smi21F	*smi97B*	−0.369	0.006
smi27E	*smi26D*	0.284	0.036
smi28E	*smi27E*	−0.319	0.018
smi51A	*smi97B*	0.275	0.042
smi61A	*smi60E*	−0.429	0.002
smi65A	*smi51A*	−0.296	0.029

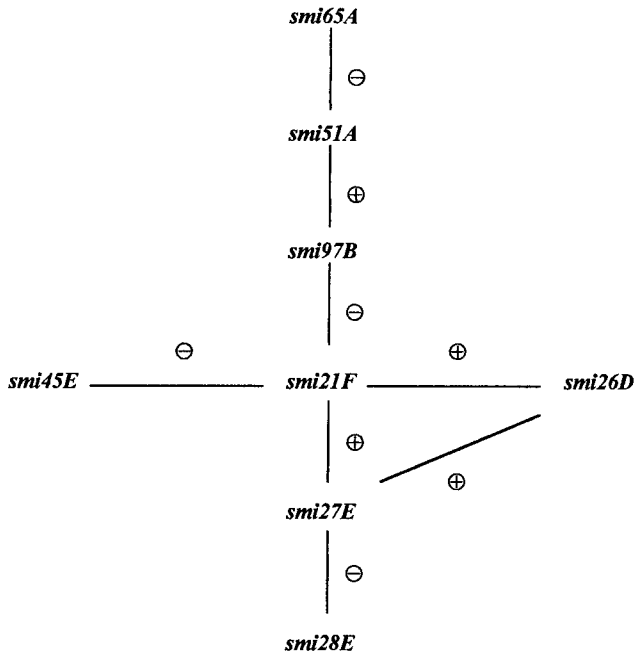

FIGURE 1.—Interaction diagram of *smi* loci. The ⊕ and ⊖ symbols indicate epistatic effects that suppress and enhance the homozygous mutant phenotype, respectively. Two loci, *smi60E* and *smi61A*, form an independent pair with a positive epistatic effect (not shown).

and MACKAY 1996). However, in this experimental design the genetic background has been standardized, and *SCA* interactions can only result from epistasis, *i.e.*, variation in heterozygous effects of a *smi* mutation depending on the genetic background with respect to other *smi* mutations.

We observed highly significant *SCA* effects ($P = 0.0025$, Table 3) for olfactory avoidance among the transheterozygote genotypes. This observation is not a scale effect. The effect of *SCA* was also highly significant if log, square root, and square transformations are applied to the data (data not shown). This suggests that epistatic interactions among loci affecting olfactory behavior are very common, because we have sampled only a small fraction of the total number of possible genotypes at 12 loci, each with two alleles (66/1728 = 3.8%). To determine which interacting mutations contributed to the overall variation in *SCA*, we determined for which transheterozygote lines *SCA* effects are significantly different from zero. The results are given in Table 4. Nine transheterozygous crosses reveal statistically significant epistatic interactions between *smi* loci. In addition, the *smi98B/smi60E* transheterozygote has an *SCA* value (−0.251) that is nearly formally significant ($P = 0.063$). In five of the nine statistically significant cases, the difference between the observed and expected avoidance scores (*SCA*) is negative; *i.e.*, the avoidance response of the double heterozygote is more mutant than would be expected given the average degrees of dominance of both parents. In four cases, the *SCA* estimates were positive, indicating better olfactory responses of the hybrid offspring than expected from the average heterozygous effects of parental mutations. It should be noted that all of the transheterozygotes show avoidance scores within wild-type range, *i.e.*, complementation, but it is the quantitative analysis of the degree of complementation that reveals epistatic effects. The negative and positive interactions are quantitative genetic analogues of mutations that enhance or suppress, respectively, the effects of other mutations affecting the same phenotype.

The observed epistatic effects are quite large; the mean of the absolute values of significant *SCA* effects is 0.34. This value is of the same magnitude as the mean of the absolute values of significant *GCA* effects (0.23), and is one-half of the environmental standard deviation. However, it is clear that these "large" quantitative effects are very subtle in absolute terms and cannot be discerned without quantitative genetic analysis of the phenotypes, or in variable genetic backgrounds. The magnitude of the epistatic effects are not necessarily correlated with the size of the homozygous mutant effects. *smi* loci with relatively small effects on olfactory behavior of homozygotes, *e.g.*, *smi21F* and *smi45E* (ANHOLT *et al.* 1996), produce large effects in double heterozygous progeny. We cannot, however, determine to what extent each locus of an interacting pair of loci contributes to the observed epistatic effect. Furthermore, we do not know to what extent the *P[lArB]* insertion limits the expression of the gene it affects. We predict, therefore, that epistatic effects will be stronger in double heterozygotes that contain null mutations, such as deletions, at the *smi* loci.

The pattern of interactions observed is interesting. Of the 12 *smi* loci, 10 interact with at least one other. Epistatic interactions between eight *smi* loci can be represented in a simple interaction diagram (Figure 1). *smi60E* and *smi61A* interact, but are independent of the others. It is possible that *smi98B* interacts with *smi60E* (the *P* value of the *SCA* is on the borderline of formal statistical significance), which would place 11 of the

12 *smi* genes in two interacting groups. It is somewhat surprising that the mutation that interacts most extensively with other *smi* mutants, *smi21F*, has itself very weak homozygous effects. The mutant phenotype of this gene is only apparent at a low concentration of benzaldehyde and is strongly sexually dimorphic (only females display aberrant olfactory responses; males are not significantly different from wild type) (ANHOLT *et al.* 1996). Yet it elicits strong interactions in transheterozygotes with four of the *smi* mutations, and in both sexes.

These loci represent only a small sample of the genes that affect olfactory behavior. The frequency with which *smi* lines were detected in our previous *P*-element mutagenesis screen indicated that ~4% of the Drosophila genome participates in shaping odor-guided behavior (ANHOLT *et al.* 1996), which corresponds to a conservative estimate of about 400 genes. Most likely, the ensemble of genes illustrated in Figure 1 is integrated into a more extensive network of interactions within the olfactory subgenome. Thus, loci that appear noninteractive, *i.e.*, *smi35A* and (possibly) *smi98B*, and loci that interact independently of the larger ensemble, *i.e.*, *smi60E* and *smi61A*, may prove to be part of a wider network of interacting genes once more olfactory genes are identified. Such extensive epistatic interactions between *smi* loci indicate that they form a complex network of genes that together shape odor-guided behavior.

In recent years, other investigators have identified olfactory mutants in *D. melanogaster*, mostly with mutations located on the *X* chromosome (VIHTELIC *et al.* 1993; WOODARD *et al.* 1992; RIESGO-ESCOVAR *et al.* 1995; LILLY and CARLSON 1989; LILLY *et al.* 1994a,b), but epistatic interactions among them and their effects on phenotypic variation have not been assessed. Our observations suggest that olfactory genes identified on the *X* chromosome might also interact within functional genetic networks and these possible interactions could also include the *smi* loci described here. However, different genetic backgrounds may render the detection of such epistatic effects more difficult than detection of epistasis among *smi* genes in a coisogenic background.

Because each of the *smi* genes used in this study is tagged by a *P* element, it will, in principle, be possible in future studies to characterize their expression products and to obtain an understanding of the molecular basis for the observed genetic interactions. Moreover, our ability to use coisogenic *P[lArB]*-insertion lines for the characterization of networks of interacting genes in the olfactory subgenome will enable the future identification of new olfactory genes by virtue of epistatic interactions with known *smi* genes. Thus, these experiments pave the road for the use of quantitative genetic analysis of subtle phenotypes as a tool for targeted gene discovery.

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